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## Genome-wide association study for morphological and hunting-behavior traits in Braque Français Type Pyrénées dogs

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<b>Abstract:</b>	<p>High-throughput genotyping offers great potential to increase our understanding of the genomic basis of canid variation. Braque Français Type Pyrénées (BRA) are smart, agile, and friendly dogs originally developed for tracking, hunting, and retrieving feathered game. On a population of 44 unrelated BRA dogs, single nucleotide polymorphism (SNP) genotype data from the CanineHD Whole-Genome Genotyping BeadChip and evaluation scores for 12 traits related to morphology and hunting performance were available. After quality filtering, 95 859 SNPs on the 38 dog autosomes (CFA) were retained. Phenotypic scores were expressed on a scale from 1 (worst) to 6 (best) and were mostly poorly to moderately correlated except for some morphological traits (e.g. <math>r = 0.81</math> between the conformation of the head and that of the eye). From GWAS, a total of 378 SNP-phenotype associations with posterior odds larger than 1 have been detected. The strongest associations were found for the eye conformation, for the skull/muzzle ratio, and for connection to the hunter. These included both new and previously identified markers and genes potentially involved with type and behavior traits in BRA. Six of the significant markers mapped within SETDB2, a gene known to be related to pointing behavior in dogs. These results advance our understanding of the genetic basis for morphology and hunting behavior in dogs and identify new variants which are potential targets for further research.</p>
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1 **Short Communication**

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4 **Genome-wide association study for morphological and hunting-behavior traits in Braque**  
5 **Français Type Pyrénées dogs**

6

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24 **Abstract**

25 High-throughput genotyping offers great potential to increase our understanding of the genomic  
26 basis of canid variation. Braque Français Type Pyrénées (BRA) are smart, agile, and friendly dogs  
27 originally developed for tracking, hunting, and retrieving feathered game. On a population of 44  
28 unrelated BRA dogs, single nucleotide polymorphism (SNP) genotype data from the CanineHD  
29 Whole-Genome Genotyping BeadChip and evaluation scores for 12 traits related to morphology  
30 and hunting performance were available. After quality filtering, 95 859 SNPs on the 38 dog  
31 autosomes (CFA) were retained. Phenotypic scores were expressed on a scale from 1 (worst) to 6  
32 (best) and were mostly poorly to moderately correlated except for some morphological traits (e.g.  $r$   
33 = 0.81 between the conformation of the head and that of the eye). From GWAS, a total of 378 SNP-  
34 phenotype associations with posterior odds larger than 1 have been detected. The strongest  
35 associations were found for the eye conformation, for the skull/muzzle ratio, and for connection to  
36 the hunter. These included both new and previously identified markers and genes potentially  
37 involved with type and behavior traits in BRA. Six of the significant markers mapped within  
38 SETDB2, a gene known to be related to pointing behavior in dogs. These results advance our  
39 understanding of the genetic basis for morphology and hunting behavior in dogs and identify new  
40 variants which are potential targets for further research.

41

42 *Keywords:* Braque Français Type Pyrénées; dogs; GWAS; behavior traits; type traits

43 Sport-hunting dogs have long been employed in assistance to hunters, reflecting strong behavioral  
44 pressure to locate and pursue quarry over great distances and variable terrain (Kim et al., 2018). At the same  
45 time, humans have bred dogs also for different morphological traits like body type, size, skull shape, coat color  
46 (Shan et al., 2021). Behavior is a complex phenotype, i.e. it is under polygenic control and susceptible to  
47 environmental influence, and it is often genetically correlated with other traits. For example, behavioral traits  
48 in dogs are known to be related to body size (McGreevy et al., 2013). To date, several genes have been  
49 identified which define breed-specific differences associated with morphological (Boyko et al., 2010; Hayward  
50 et al., 2016; Vaysse et al., 2011) and hunting performance traits (Akkad et al., 2015; Kim et al., 2018).

51 Braque Français Type Pyrénées (BRA) dogs originate from an old type of gundog originally developed  
52 for detecting, tracking, pointing and retrieving feathered game during hunting. Small size, high head posture,  
53 smart, agile, energetic, indomitable temperament, good water confidence, collaborative in hunting, affectionate  
54 and playful disposition are typical characteristics of this breed (Di Gerlando et al., 2019; Mastrangelo et al.,  
55 2018a, 2018b).

56 This study used evaluation scores for 12 traits related to morphology and hunting behavior phenotypes  
57 to conduct a GWAS in the BRA breed aimed at uncovering potentially associated genes.

58 A total of forty eight-months-old dogs (25 females, 19 males) registered in the Italian Kennel Club  
59 “ENCI were used. Morphological and hunting-behavior traits were scored by breed-expert judges from the  
60 Federation Cynologique Internationale (<https://www.fci.be/en/>) and ranged from 1 (poorest evaluation) to 6  
61 (best evaluation). Traits and score ranges are summarized in Supplementary Table S1.

62 Genotyping data were retrieved from a previous study (Mastrangelo et al., 2018a). After excluding  
63 SNPs that were unmapped, on the sex chromosomes, with call-rate < 95% and MAF (minor allele frequency)  
64 < 5%, 95,859 SNPs were retained for the analysis. PLINK v.1.9 was used to process SNP data (Chang et al.,  
65 2015). Missing SNP genotypes were imputed with LHCI (localized haplotype clustering imputation: Beagle  
66 v.4.1, Browning and Browning (2016)). SNP positions from the CanFam3.1 genome sequence assembly (GCF  
67 000002285.3) were used.

68 The association between SNP genotypes and morphological or hunting-behavior traits was tested using  
69 the following linear mixed model:

$$70 \quad \mathbf{y} = \mathbf{Xb} + \mathbf{Sa} + \mathbf{Zu} + \mathbf{e} \quad (1)$$

71

72 where:  $\mathbf{y}$  is the vector of phenotypes;  $\mathbf{b}$  is the vector of systematic effects, i.e. intercept and sex;  $\alpha$  is  
73 the SNP (fixed) effect;  $\mathbf{u}$  is the vector of polygenic (random) effects;  $\mathbf{e}$  is the vector of residuals.  $\mathbf{X}$ ,  $\mathbf{S}$  and  $\mathbf{Z}$   
74 are the corresponding design matrices, relating records to sex, SNP genotypes and dogs. SNP genotypes were  
75 coded as the number of copies of the minor allele (0/1/2).  $\text{Var}(\mathbf{y}) = \text{Var}(\mathbf{u}) + \text{Var}(\mathbf{e}) = \mathbf{K}\sigma_{\mathbf{u}}^2 + \mathbf{I}\sigma_{\mathbf{e}}^2$ , where  $\mathbf{K}$  is  
76 the matrix of genomic relatedness (VanRaden, 2008),  $\mathbf{I}$  is the identity matrix, and  $\sigma_{\mathbf{u}}^2$  and  $\sigma_{\mathbf{e}}^2$  are the additive  
77 genetic and residual variances. The model in equation (1) was run using the R package GenABEL v.1.8.0  
78 (Aulchenko et al., 2007), separately for each SNP and each phenotype. To identify significant associations, we  
79 estimated the posterior odds of true association (Burton et al., 2007), based on the GWAS power and the prior  
80 probability of association. For 20% heritability, sample size of 50 individuals and type I error (unadjusted p-  
81 value threshold) equal to 0.001, the expected statistical power of a GWAS experiment is 60% (Visscher et al.,  
82 2017). Assuming 100 QTLs controlling the phenotypes, on average, the posterior odds of association were  
83 estimated as:

$$84 \quad \text{Posterior odds} = \frac{\text{prior probability} \cdot \text{power}}{p - \text{value}}$$

85

86 where the prior probability is given by the ratio of the expected number of QTL for the trait (assumed  
87 as 100) and the number of SNPs tested (95 859). SNP genotype-phenotype associations with posterior odds >  
88 1 (higher odds that the SNP is associated with the phenotypes rather than it is not) were considered significant  
89 results. Genomic regions showing significant results were further explored to identify candidate genes, using  
90 the Genome Data Viewer developed by NCBI  
91 ([https://www.ncbi.nlm.nih.gov/genome/gdv/browser/genome/?id=GCF\\_000002285.3](https://www.ncbi.nlm.nih.gov/genome/gdv/browser/genome/?id=GCF_000002285.3)).

92 Traits descriptives in male and female dogs are reported in Supplementary Table S2. The 12 traits  
93 showed mostly poor or moderately positive linear correlations (Figure 1), exceptions were the conformation  
94 of the eye and that of the head ( $r = 0.81$ ). The only relevant negative correlations were found for the height at  
95 withers with game retrieval ability ( $r = -0.30$ ) and aquaticity ( $r = -0.32$ ).

96 From GWAS (equation (1)), 378 SNPs with posterior odds larger than 1 have been detected (Figures  
97 2 and 3, Supplementary Table S3). The trait with the largest number of markers was the connection with the

98 hunter (n=144), followed by the skull/muzzle ratio (n=54) and aquaticity (n=49). Overall, approximately 130  
99 genes have been identified. Table 1 lists the 18 most significant markers (posterior odds of association  $\geq 5$ ).

100 In this communication, we focus on a few selected genes related to traits involved in morphological  
101 and hunting-behavior traits. For the aptitude to collaborate (collaboration), we identified 15 markers on 7  
102 chromosomes. The SNP BICF2P417531 (CFA 18) mapped within *SHANK2*, a gene involved in hyperactivity  
103 and panic response in Terrier dogs (Kim et al., 2018). For the direction of the skull-facial axis (cranio-facial),  
104 two SNPs on CFA 4 mapped in or close to *ZMIZ1*, a gene involved in a neuro-development disorder with  
105 dysmorphic facies and distal skeletal anomalies (*NEDDFSA*) (Lu et al., 2022). As for the shape and position  
106 of the eye, SNP BICF2S23626288 was found to lie within *DNAH2*, a gene from the gene-set previously  
107 reported to show statistically significant differences from expression analysis related to retinal degeneration in  
108 several dog breeds (Goldstein, 2014). For the connection with the hunter (hunter), six markers (on CFA 22)  
109 mapped within *SETDB2*, a gene that may contribute to pointing behavior (Akkad et al., 2015). Interestingly,  
110 *SETDB2* is also associated with the establishment of left-right asymmetry. Since the degree of motor laterality  
111 has been identified as a key predictor of success in guide-dog training, it can be speculated that variability in  
112 *SETDB2* might be associated with learning abilities that in turn may influence the establishment of pointing  
113 behavior (Akkad et al., 2015; Vaysse et al., 2011). Additional significant markers on CFA 22 (7 and 9 SNPs)  
114 identified candidate genes (*FNDC3A* and *CYSLTR2*) previously reported to be under selection in dogs with a  
115 breeding-for-hunting background (Vaysse et al., 2011). Other genes on CFA 22 linked to markers associated  
116 with the 'hunter' trait include *KPNA3*, *EBPL*, *RCBTB1*, *CAB39L*, *CDACD1*, *MLNR* and *RCBTB2*. These genes  
117 have been identified within regions under selections in hunting dogs (Akkad et al., 2015; Perfilyeva et al.,  
118 2023; Yu et al., 2022). Among these, *CAB39L* is a candidate gene that affects the running speed and endurance  
119 in dogs (Perfilyeva et al., 2023). Finally, the SNP BICF2G630437454 on CFA 15 and associated with aquaticity  
120 (water), mapped near ( $< 100$  Kb) *ACSS3*, a gene that is hypothesized to contribute to the development of  
121 temperament in dogs (Shan et al., 2021).

122 In this study, we have identified associations between genetic variants and morphological and hunting-  
123 behavior phenotypes in BRA. These included new and yet unreported, as well as previously identified, markers  
124 and genes potentially involved with type and behavior traits in *Canis familiaris*.

125            However, it must be acknowledged that this is a small-sized study, with limitations related to the  
126 statistical power of the analysis and to the robustness and generalizability of results. On the other hand, it is  
127 true that dog genomes have undergone strong artificial selection with increased haplotype homozygosity and  
128 linkage disequilibrium, and therefore, compared to human studies, GWAS with relatively small sample sizes  
129 can produce good results in dogs (Dodman et al., 2010; Lindblad-Toh et al., 2005). These results advance our  
130 understanding of the genetic basis for morphology and hunting behavior in dogs and identify new variants  
131 which are potential targets for further research.

132

### 133 **Conflict of interest statement**

134            None of the authors of this paper has a financial or personal relationship with other people or  
135 organisations that could inappropriately influence or bias the content of the paper.

136

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139 research and contributing the data used for this work. The funders had no role in study design.

140

### 141 **Disclosure statement**

142            No potential conflict of interest was reported by the authors.

143

### 144 **Data availability**

145            The data that support the findings of this study are available upon request from the corresponding  
146 author.

147

### 148 **Ethical statement**

149            No experimental studies were conducted on these animals. Phenotypic records were provided by the  
150 Italian Club Bracco Francese (officially recognized by the ENCI Italian Kennel Club- in 2006). Genotyping  
151 data for the dogs included in this study were retrieved from a previous. Therefore, no ethical approval was  
152 required.

153

154

155 **Appendix A: Supplementary material**

156 **Supplementary Table S1:** Definition of the semi-quantitative scores used to phenotype Braque Français type  
157 Pyrenées dogs for hunting behaviour (top) and morphological (bottom) traits.

158 **Supplementary Table S2:** Descriptive statistics of the morphological and hunting-behaviour phenotypes  
159 analysed in this study in males (M) and females (F).

160 **Supplementary Table S3:** List of all 378 SNP-phenotype associations with posterior odds larger than 1 (235  
161 for hunting-behavior traits and 155 for morphological traits).

162



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217

218 **Table 1**

219 SNP-phenotype associations with posterior odds of true association larger than 5. Associations are first grouped by phenotype category (behavior,  
 220 morphology), then by decreasing posterior odds of association.

221

Type	Trait	SNP	<i>p</i> -value	Post. odds	Chrom	Pos (bps)	Gene_within	Gene_near
behavior	hunter	BICF2G630598544	3.64E-05	17.202	32	10959734		<i>SPARCL1</i>
behavior	collaboration	BICF2G630510152	7.58E-05	8.256	24	10904518		
behavior	hunter	BICF2S23627579	0.0001218025262	5.139	12	46920426	<i>CFAP206</i>	
behavior	hunter	BICF2S23423118	0.0001218025262	5.139	12	47109734		<i>ORC3</i>
behavior	water	BICF2S23550231	0.0001220584142	5.128	10	65541626	<i>LOC111097793</i>	
morphology	eye	BICF2P153003	1.35E-05	46.486	5	33872506	<i>STX8</i>	
morphology	skull.muzzle	BICF2P289826	3.27E-05	19.138	29	21219943		<i>KCNB2</i>
morphology	skull.muzzle	BICF2G630623555	3.61E-05	17.321	29	21475891		
morphology	cranio.facial	BICF2P347412	5.22E-05	11.981	6	74946630	<i>PTGER3</i>	
morphology	head	BICF2S23410489	6.48E-05	9.654	5	27669801		
morphology	cranio.facial	BICF2G630198340	7.17E-05	8.730	26	33943479	<i>PCDH15</i>	
morphology	eye	BICF2P1303880	7.24E-05	8.643	13	13824164	<i>LOC100686713</i>	
morphology	skull.muzzle	BICF2S23656452	7.31E-05	8.564	6	18547621	<i>LOC111096272</i>	
morphology	type	BICF2G63091624	7.94E-05	7.882	22	59638332	<i>LOC111091721</i>	
morphology	skull.muzzle	BICF2S2414260	8.69E-05	7.200	31	30712199		<i>MIR802</i>
morphology	cranio.facial	BICF2P230586	0.0001036740769	6.037	2	15363081	<i>ZEB1</i>	
morphology	cranio.facial	BICF2P501332	0.0001061773921	5.895	6	74959030	<i>PTGER3</i>	
morphology	cranio.facial	BICF2P501333	0.0001061773921	5.895	6	74959125	<i>PTGER3</i>	

222

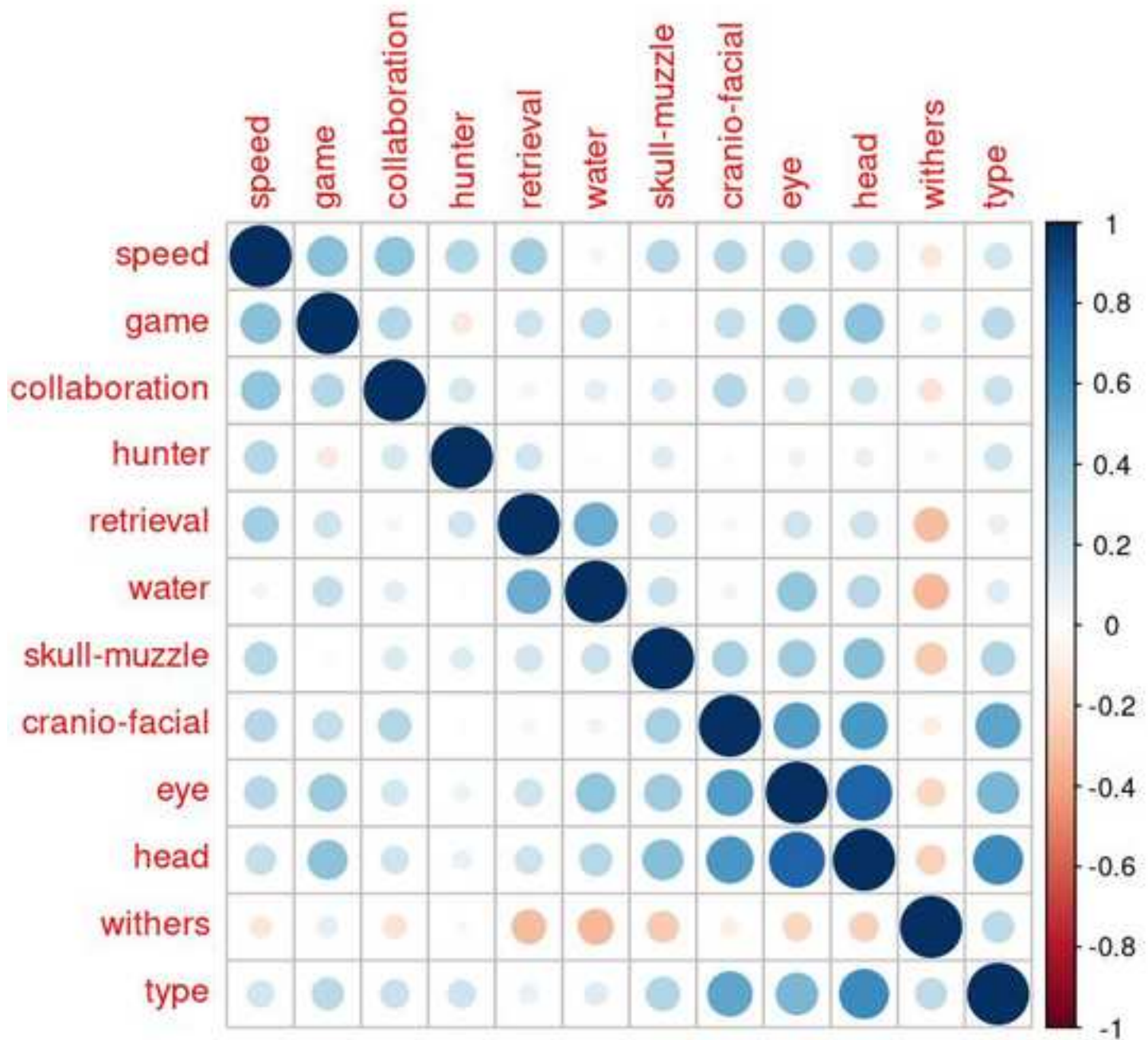
223 **Figure legend.**

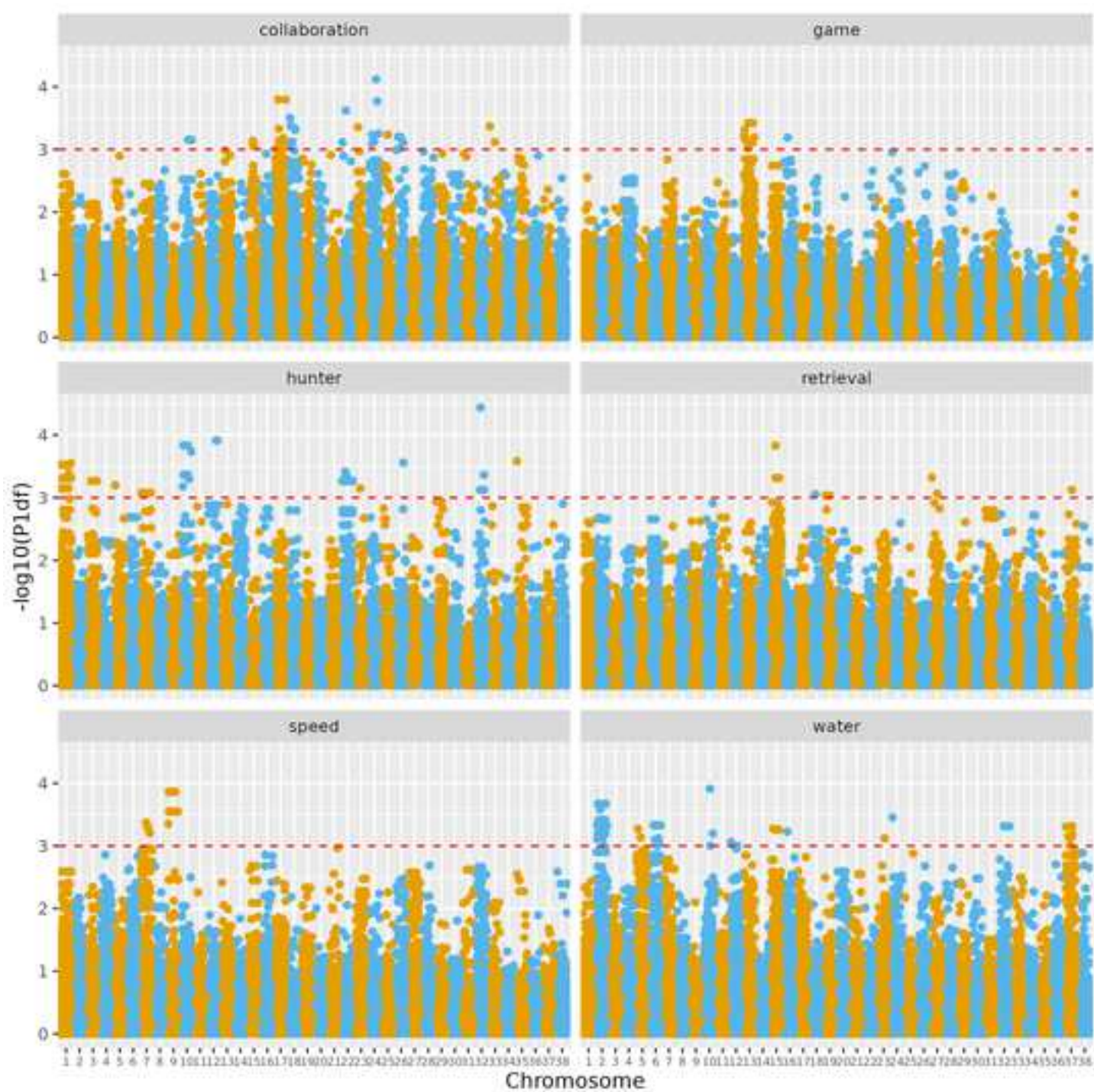
224 **Fig. 1.** Correlation plot of the hunting-behavior and morphological traits measured on Braque  
225 Français type Pyrénées dogs. Shades of blue indicate positive correlations, shade of red indicate  
226 negative correlations (the darker the color, the stronger the correlation).

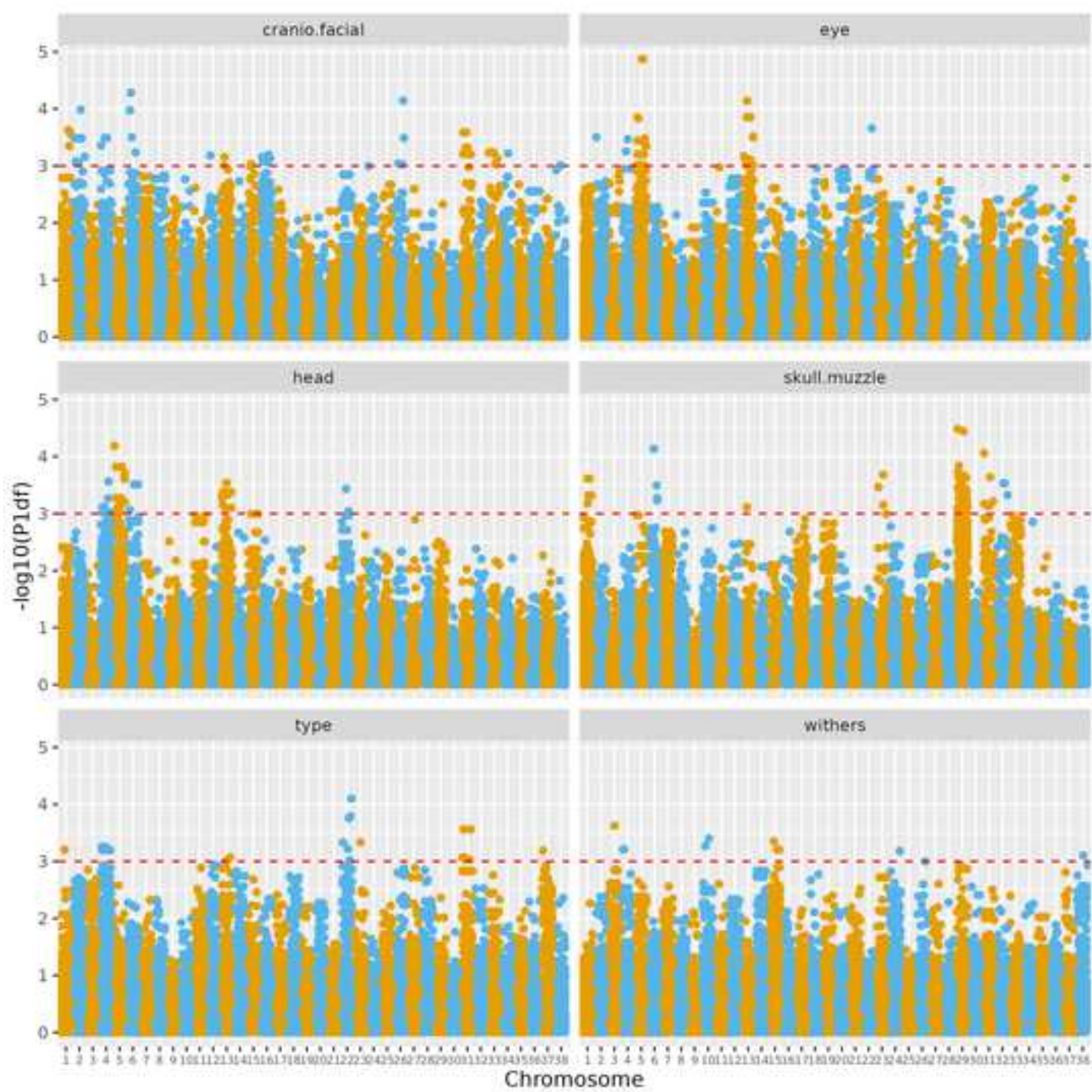
227 **Fig. 2.** Manhattan plots of GWAS results for the six hunting behavior traits in Braque Français dogs.

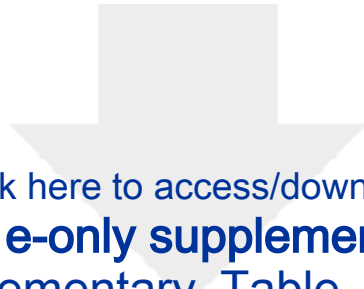
228 **Fig. 3.** Manhattan plots of GWAS results for the six morphology traits in Braque Français dogs.

229



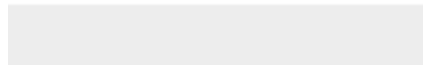
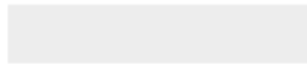




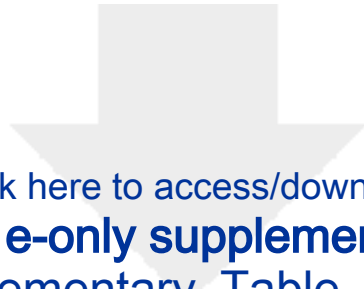


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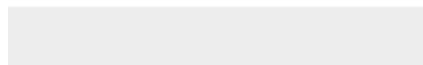


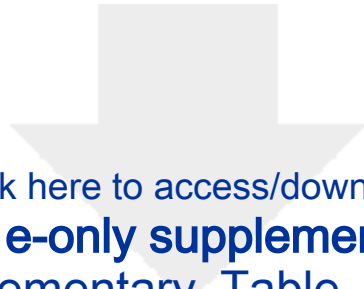




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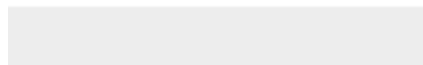
**Optional e-only supplementary files**  
Supplementary\_Table\_S2.pdf





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**Optional e-only supplementary files**  
Supplementary\_Table\_S3.pdf



- GWAS for type and hunting-behavior traits in Braque Français Type Pyrénées dogs
- 378 SNP-phenotype associations with posterior odds larger than 1 have been detected
- strongest associations for eye conformation, skull/muzzle ratio, connection to hunter
- six significant SNPs within *SETDB2*, a gene related to pointing behavior in dogs

Dear Editor(s) at The Veterinary Journal,

We are submitting our article with title "Genome-wide association study for morphological and hunting-behavior traits in Braque Français Type Pyrénées dogs" as Short Communication to be considered by your journal. This is a pilot study on the genetic background of morphology and hunting-behavior traits in a relatively understudied canine population. As pilot study, only a limited number of subjects was included, yet we believe that this work can be interesting given the novel traits and population addressed, and the rigorous and clearly described experimental approach.

Our Short Communication consists of about 1,300 words (excluding abstract and references): please let us know if we need to make an extra effort to shorten the text and/or amend our submission in any other ways.

Sincerely,

Filippo Biscarini  
Italian National Council for Research (IBBA-CNR)  
e-mail: [filippo.biscarini@ibba.cnr.it](mailto:filippo.biscarini@ibba.cnr.it)